Wheat Protection from Root Rot Caused by *Fusarium culmorum* Using Silver Nanoparticles

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Summary: Nanomaterials have a positive impact on agriculture. Silver nanoparticles were used to enhance seed germination, plant growth, and as antimicrobial agents to control plant diseases. In the present study, the effectiveness of silver nanoparticles on seed germination of wheat, growth parameters, and control of root rot disease caused by *Fusarium culmorum* were examined. Three different concentrations (10, 20, and 40 mg/l) were used. Exposure to AgNPs had no significant effects on the seed germination at 10 mg/l and 20 mg/l while at 40 mg/l significant effects were observed compared to that in the control (untreated). Germination was highest (86%) after exposure to 20 mg/l of AgNPs. AgNPs, at all concentrations tested, had significant effects on the pre-emergence, post-emergence and survival as compared to the control (infected and untreated with AgNPs); the highest effects were observed after exposure to 40 mg/l of AgNPs (15, 10, and 75% respectively). Additionally, our results indicate that plant height, fresh weight, and dry weight were significantly increased at 10 mg/l (23.4 cm, 6 g, and 1.45 g), and 20 mg/l (27.3 cm, 7.5 g, and 1.98 g) respectively, compared with that of the control. However, higher concentration (40 mg/l) of AgNPs decreased the growth parameters.

Key words: Silver nanoparticles, *F. culmorum*, Wheat, Seed germination, Growth parameters.

Introduction

Cereal grains and its products have important sources of protein and energy [1]. Wheat is considered an essential crop, and is ranked third, after maize and rice. World production of wheat in 2013 was 713,182,914 tons; area harvested was 218,460,701 Ha, and yield was 32,646 Ha [2]. Diseases of wheat caused by *Fusarium*, includes a number of species, but mostly *F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. poae*, and *M. nivale* [3-8]. The application of nanotechnology such as food packaging, food security, the use of nanoparticles in biosensors for detection pathogens and contaminants [9] water refinin, wastewater and environmental treatment [10]. In the agriculture field, Considered of using nanomaterials is relatively new and needs more studies However, these materials are used in agriculture production and crop protection. [10]. Nanotechnology can be applied in precision farming to increase crop yields and minimizing fertilizers, pesticides, herbicides [11]. Other alternatives to agrochemicals are biopesticides and biofertilizers. Utilization of nanotechnology could lead to a reduction in pesticides by 70–80%, resulting in a significant reduction in cost and quantity of pesticides [12]. Silver (Ag) hane been more toxic to various microorganisms than many other metals in the following order: Ag, Hg, Cu, Cd, Cr, Pb, Co, Au, Zn, Fe, Mn, Mo, Sn [13]. Many studies have used silver nanoparticles as antimicrobial agents against microorganisms such as *E. coli* [14-16], yeast and *Staphylococcus aureus* [16]. Other microbes include *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Fusarium oxysporum*, *Curvularia lunata*, *Rhizopus arrhizus*, *Aspergillus niger*, and *Aspergillus flavus* [16]. The efficiency of AgNPs could have estimated by the fact that they kill approximately 650 kind of pathogenic microbes in a very stumpy time [17].

Experimental

Isolation of F. culmorum

Wheat roots were cut into small fractions, washed completely with faucet water, then sterilized with sodium hypochlorite solution (equivalent to 5% chlorine) for 1 min. thereafter, washed several times by sterile distilled water and dried finaly placed on potato dextrose agar media and incubated at 25°C for 7 days. Single-spore technique was used for purification. All the obtained isolates were microscopically identified according to the morphological features [18]. The identification of the isolates was carried out by the Regional Center of Fungi and their Applications, Al-Azhar University, Cairo.

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Preparation of Inoculum Suspension and Soil Infestation

Fungal inocula were prepared by inoculating potato dextrose medium in a 250 ml flask with 5-mm disk of a 7-day-old culture of *F. culmorum*, and then incubated at 27 ± 2°C for 15 days. Pots of 20 cm diameter were used, which were always sterilized by immersing in 5% formalin for 15 min and then air dried for 5 days. Soil infestation was carried out by adding the fungal inoculum to the sterilized soil at 3% of soil weight [19]**.** Fungal inocula were thoroughly mixed with the soil and regularly watered every day for a week before planting to ensure even distribution and growth of the fungus.

Preparation of Silver Nanoparticles

Silver nanoparticles (AgNPs) were produced using *Aspergillus niger* (STRAIN KSU-12) and characterized by UV–Vis spectrophotometry, Fourier transform infrared spectroscopy (FTIR) have been confirmed that amino acid and peptides have formed a coat covering the silver nanoparticles to prevent agglomeration. In the analysis by energy dispersive spectroscopy (EDS), the signals for N and O indicate the presence of proteins as a capping material on the surface of silver nanoparticles ,transmission electron microscopy (TEM) and scanning electron microscopy (SEM), as described in our previous study [20]. These nanoparticles were used for the current study.

Effect of Silver Nanoparticles on Seed Germination Percentages

Wheat seeds were purchased from the local market. The seeds were washed with deionized water, sterilized in a 5% sodium hypochlorite solution for 1 min, followed by rinsing several times with deionized water. Filter papers were used for germination in petri dishes (10 seeds / dish). Silver nanoparticles in different concentrations (0, 10, 20, and 40 mg/l) were prepared directly in deionized water. Each concentration was prepared in three replicates. Seeds were placed in deionized water (check) and 10, 20, and 40 mg/l of AgNP solutions and shaken softly for one hour as described elsewhere [21]. Ten seeds were evenly spaced on the surface of the filter paper in each Petri dish and 5 ml of each concentration of AgNP suspensions were added to the filter paper. The dishes were wrapped and kept on a lab bench at room temperature (30 ± 5°C). The experiment was performed with three replicates. The number of germinated seeds will be counted after 5 days and germination percentage was calculated using the following equations [22]

Germination Percentage = (T/n) × 100

where T= total number of seeds were sprout, n= total number of seeds were used.

Effect of Soaking Wheat Seeds in Silver Nanoparticles on Controlling Root Rot Disease and Some Growth Parameters

Wheat seeds were soaked in the AgNP solutions for 1 h before planting. Three different concentrations (10, 20, and 40 mg/l) were applied. The wetted seeds were spread out in a thin layer and left about 24 h, and then they were sown in pathogen-infested potted soils at the rate of 10 seeds/pot at a depth of 1 cm. Wheat seeds were planted at rate of 10 seeds/pot containing soil infested with *F. culmorum*, which were used as control. Three pots were used for each treatment as replicates. Data were recorded by examining of pre- and post-emergence damping-off and survival of wheat plants recorded after 15, 30, and 45 days of sowing by using the following formula as described elsewhere [23]. Plant length and plant weight were recorded.

$$\text{Pre-emergence (\%)} = \frac{\text{Total No. of ungerminated seeds} \times 100}{\text{Total No. of planted seeds}}$$

$$\text{Post-emergence (\%)} = \frac{\text{Total No. of rotted seedlings} \times 100}{\text{Total No. of planted seeds}}$$

$$\text{Survived seedlings (\%)} = \frac{\text{Total No. of survived seedlings} \times 100}{\text{Total no. of planted seeds}}$$

Statistical Analysis

Data analysis was performed using SAS software [24]. All data were expressed as mean ± standard deviation

Results and Discussion

Effect of Silver Nanoparticles on Seed Germination Percentages

Transmission electron microscopy (TEM) as described nanoparticles was 5–35 nm in size (Fig. 1).

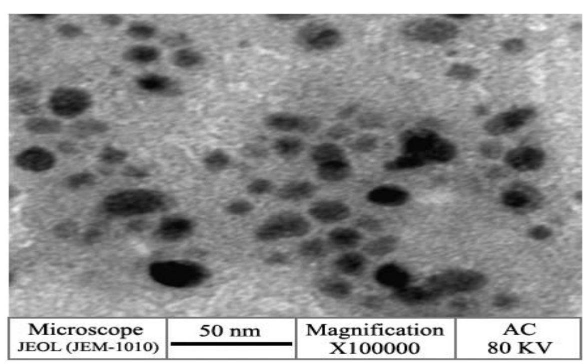

Fig. 1: Transmission Electron Microscopy (TEM) images of synthesized silver nanoparticles

Germination is important for determining the density of the final plant [26]. This study showed that effect of AgNPs with three different concentrations on germination percentages. Our results in Table-1 indicated that exposure to AgNPs had no significant effects on the germination at 10 mg/l and 20 mg/l, while at 40 mg/l was found to have significant effects as compared to the control (untreated). The highest germination percentage (86%) was observed after exposure to 20 mg/l of AgNPs. These findings are in agreement with some previous studies [27-30], while there was no agreement with other studies [25, 31, 32]. No effect on germination on wheat seed at lower concentrations 25 to 75 ppm or caused significant reduction in germination with 100-150 ppm [33]. Similar effects have been reported in other crops Baccopa monnieri and zucchini plant [34, 35]. Inhibitory effects were observed in aqueous suspensions at 10 mg / l for Ag [36] .The toxicity of AgNPs and AgNO to the seeds of Brassica juncea var. Varuna. We found that an increase in the concentration of AgNO reduces the shoot length and root length, maximum toxicity was observed at 500 mg/l [37] .This may be due to selective permeability of the seed coats [38]. Decreasing the toxicity of AgNP, which can cause lower exposure to seeds and seedlings, may be due to aggregation by ligands [39]. A variety of AgNPs effective on seed germination has been dependent on nanoparticle properties and plant type [32]. Seed germination may be decreased at higher concentrations of AgNPs, possibly due to penetration of the nanoparticles through the seed coat, thereby having an effect on the process of seed germination. It is possible that nanoparticles may cause increased water absorption by seeds [40], increased activity of nitrate reductase enzyme, increased levels of seed antioxidants [41], reduction of the levels of H_2O_2, leading to reduced antioxidant stress, and increased activities of some enzymes [42], These changes lead to increase in growth parameters.

Table-1: Effect of silver nanoparticles on seeds germination percentages at 10, 20, and 40 mg/l.

Concentration (mg/l)	Germination %	SD
0.0	81.3	±0.57
10.0	83.3	±.040
20.0	86.0	±0.68
40.0	70.0	±1.76

Effect of Soaking Wheat Seeds in Silver Nanoparticles on Controlling Root Rot Disease

Data in Table-2 show the toxic effects of AgNPs at different concentrations on root rot disease caused by *F. culmorum*. Our results in Table-2 indicated that exposure to any tested concentration of AgNPs had significant effects on the pre-emergence, post-emergence and survival as compared to the control (infected and untreated with AgNPs). The highest effects were observed after exposure to 40 mg/l of AgNPs (15, 10, and 75% respectively). Release of Ag ions from nanoparticles and the free radicals generated bring about the toxic effects of AgNPs [43]. Mechanism of inhibition of Ag^+ ions on microorganisms is by blocking DNA replication [44], inactivation of ribosomal protein expression, as well as inhibition of enzymes essential for ATP production [45]. Silver nanoparticles cause effects on membrane enzymes [46]. Metallic silver is relatively unreactive, while AgNPs are highly reactive as they generate Ag^+ ions [47]. Ability of nanoparticles to penetrate into microbial cells, even at lower concentrations would be sufficient for microbial control. This would be more effective, especially for some organisms, which are less sensitive to antibiotics [48]. Silver nanoparticles disrupt transport systems including ion efflux, which can cause rapid accumulation of Ag^+ ions, leading to disturbance in cellular processes such as metabolism and respiration [48]. In addition, Ag^+ ions damage to nucleic acids, proteins, and lipids by producing reactive oxygen species, which are destructive to cells [49]. Preventive applications of AgNPs work better before fungal isolates penetrate and colonize within the plant tissue [43]. The most effective way is direct application of nanoparticles in the soil on seeds to protect plants from pathogen invasion [50].

Table-2: Effect of soaking wheat seeds in silver nanoparticles on controlling root rot disease.

Concentration (mg/l)	Pre%	SD	Post%	SD	Survival%	SD
0.0	46.2	±0.60	30	1.76	23.8	±1.59
10.0	20.4	±0.50	18.9	0.80	60.7	±1.08
20.0	17.5	±0.26	14.1	0.61	68.4	±0.75
40.0	15.0	±0.55	10.0	0.46	75.0	±0.85

Effect of Soaking Wheat Seeds in Silver Nanoparticles on Some Growth Parameters

Data in Table-3 show that effect of silver nanoparticles on plant height, fresh weight, and dry weight. Our results indicate that plant height, fresh weight and dry weight were significantly increased at 10 mg/l (23.4 cm, 6 g, and 1.45 g), and at 20 mg/l (27.3 cm, 7.5 g, and 1.98 g) respectively, compared with that of the control. However, higher concentration (40 mg/l) of AgNPs decreased the growth parameters. It was observed that higher concentrations of nanoparticles had adverse effect. Inhibition of seed germination and seedling elongation has been found to be highly dependent on both plant type and properties of nanoparticles [30]. During penetration of AgNPs inside the root cell, the cell wall could be damaged and vacuoles may be formed due to the penetrations of larger particles entering through small pores of cell walls. It has been reported that AgNP treatment generally affected the shoot and root growth, rather than seed germination [27].

Table-3: Effect of soaking wheat seeds in silver nanoparticles on some growth parameters.

Concentration (mg/l)	Plant height (cm)	SD	Fresh weight (g)	SD	Dry weight (g)	SD
0.0	22.5	0.50	5.2	0.47	1.33	±0.47
10.0	23.4	0.46	6.0	0.52	1.54	±0.13
20.0	27.3	0.85	7.5	0.40	1.98	±0.05
40.0	18.8	0.57	4.4	0.46	1.17	±0.25

Conclusion

Silver nanoparticles may have significant applications in agriculture by inhibiting harmful fungi as an alternative fungicide that may improve sustainable agriculture. Thus, nanoparticle treated seeds can be used to reduce the use of chemicals. The exposure to AgNPs had no significant effects on the germination percentage at 10 mg/l and 20 mg/l, whereas at 40 mg/l was found be significantly effective. However, any tested concentration of AgNPs had significant effects on the pre-emergence, post-emergence, survival, plant height, fresh weight, and dry weight as compared to control.

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References

1. C. M. Placinta, J. P. F. D'Mello and A. M. C. Macdonald, A Review of Worldwide Contamination of Cereal Grains and Animal Feed with Fusarium Mycotoxins, *Anim Feed Sci Tech*., **78**, 21 (1999).
2. FAO http://faostat.fao.org/site/339/default.aspx, (2013).
3. I. Heidi and G. Abo-Elnaga, Biological Control of Damping Off and Root Rot of Wheat and Sugar Beet with *Trichoderma harzianum, Plant Pathol. J*., **11**, 25 (2012).
4. K. Hudec and T. Roháčik, *Fusarium* spp. and *Microdochium nivale* Infestation of Asymptomatic Wheat Kernels in Slovakia, *Cereal Res. Commun*., **31**, 415 (2003).
5. B. Tunali, I. Ozseven, O. Buyuk, D. Erdurmus and A. Demirci, Fusarium Head Blight and Deoxynivalenol Accumulation of Wheat in Marmara Region and Reactions of Wheat Cultivars and Lines to *F. graminearum* and *F. culmorum, Plant Pathol. J*., **5**, 150 (2006 b).
6. B. J. Tunali, J. Nicol, F. Y. Erol and G. Altiparmak, Pathogenicity of Turkish Crown and Head Scab Isolates on Steam Bases on Winter Wheat Under Greenhouse Conditions, *Plant Pathol. J*., **5**, 143 (2006 a).
7. J. Kohl, B. H. de Dehaas, P. Kastelein, S. L. Burgers and C. Waalwijk, Population Dynamics of Fusarium spp. *Microdochium nivale* in Crops and Crop Residues of Winter Wheat, *Phytopathol*., **79**, 971 (2007).
8. G. Kammoun, K. Gargouri and G. Samia, Pathogenicity and Aggressivenss of Fusarium and Microdochium on Wheat Seedling Under Controlled Conditions, *Tunisian J. Plant Protect*., **4**, 135 (2009).
9. J. Jampílek and K. Kráľová, Application of Nanotechnology in Agriculture and Food Industry, its Prospects and Risks, *Ecol. Chem. Eng.*, S **22**, 321 (2015).
10. L. R. Khot, S. Sankaran, J. M. Maja, R. Ehsani and E. W. Schuster, Applications of Nanomaterials in Agricultural Production and Crop Protection: A review, *Crop Protect.*, **35**, 64 (2012).
11. D. Rickman, J. C. Luvall, J. Shaw, P. Mask, D. Kissel and D. Sullivan (1999) Precision Agriculture, Changing the Face of Farming. Geotimes Feature Article. www.ghcc.msfc.nasa.gove/precisionag/ Accessed 19 November, 2011.
12. K. Murphy, Nanotechnology: Agriculture’s Next “Industrial” Revolution, V. T. Williston, Financial Partner, Yankee Farm Credit, ACA, Spring, **8**, 3 (2008).
13. G. J. Zhao and S. E. Stevens, Multiple Parameters for the Comprehensive Evaluation of the Susceptibility of *Escherichia coli* to the Silver Ion, *Bimetals*, **11**, 27 (1998).
14. S. W. Kim, K. S. Kim, K. Lamsal, Y. J. Kim, S. B. Kim, M. Jung, S. J. Sim, H. S. Kim, S. J. Chang, J. K. Kim and Y. S. Lee, An In Vitro Study of the Antifungal Effect of Silver Nanoparticles on Oak Wilt Pathogen Raffaelea sp., *J. Microbiol. Biotechnol*., **19**, 760 (2009).
15. J. S. Kim, E. Kuk, K. N. Yu, J. H. Kim, S. J. Park, H. J. Lee, S. H. Kim, Y. K. Park, Y. H. Park, C. Y. Hwang, Y. K. Kim, Y. S. Lee, D. H. Jeongand and M. H. Cho, Antimicrobial Effects of Silver Nanoparticles, *Nanomedicine*, **3**, 95 (2007).
16. N. Savithramma, R. M. Linga and D. Suhrulatha, Screening of Medicinal Plants for Secondary

Metabolites, *Middle East J. Sci. Res*, **8**, 579 (2011).
17. S. Shahrokh and G. Emtiazi, Toxicity and Unusual Biological Behavior of Nanosilver on Gram Positive and Negative Bacteria Assayed by Microtiter-Plate, *Eur. J. Biol. Sci.*, ***1*** *, 28-31, (2009).*
18. G. M. Waterhouse, The genus *Phytophthora* diagnosis (or description) and figures from the original papers. Miscellaneous Publication, No.12, (1956).
19. M. M. Mazen, Ph.D. Thesis, *Resistance Induction Against Diseases of Faba Bean Crop*, Faculty of Agriculture, Suez Canal University, (2004).
20. A. R. M. Abd El-Aziz, M. R. Al-Othman, S. A. Al-Sohaibani, M. A. Mahmoud and S. R. M. Sayed, Screening of Medicinal Plants for Secondary Metabolites, *Digest J. of Nanomaterials and Biostructures*, **7**, 1491 (2012).
21. W. WoŸniak, Physical Results of Barley Grain Moisture Treatment (in Polish), *Acta Agrophysica*, **110,** 235 (2004).
22. S. Ruan, Q. Xue and K. Tylkowska, The Influence of Priming on Germination of Rice *Oryzo sativa* L. Seeds and Seedling Emergence and Performance in Flooded Soil, *SeedSci. Technol*., **30,** 61 (2002).
23. M. M. M. Ragab, M. M. Saber, S. A. El-Morsy, A. R. M. Abd El-Aziz, Induction of Systemic Resistance Against Root Rot of Basil Using Some Chemical Inducers, *Egypt J. Phytopathol*., **37,** 59 (2009).
24. SAS, Statistical system, *Release 6.03* edition, *SAS institute. Inc. Carry, North Carolina, USA (1987).*
25. N. Savithramma, S. Ankanna and G. Bhumi, Effect of Nanoparticles on Seed Germination and Seedling Growth of *Boswellia ovalifoliolata* an Endemic and Endangered Medicinal Tree Taxon, *Nano. Vision*, **2**, 61 (2012).
26. R. Z. Baalbaki, R. A. Zurayk, S. N. Bleik and A. Talhuk, Germination and Seedling Development of Drought Susceptible Wheat Under Moisture Stress, *Seed Sci. Technol*., **17**, 291 (1990).
27. L. Yin, B. P. Colman, B. M. McGill, J. P. Wright and E. S. Bernhardt, Effects of Silver Nanoparticle Exposure on Germination and Early Growth of Eleven Wetland Plants, *PLOS ONE*, **7,** e47674 (2012).
28. D. H. Lin and B. S. Xing, Pytotoxicity of Nanoparticles: Inhibition of Seed Germination and Root Growth, *Environ Pollut*, **150**, 243 (2007).
29. M. R. Castiglione and R. Cremonine, Nanoparticles and Higher Plants, *Caryologia*, **62**, 161 (2009).
30. E. A. Abdel-Azeem and B. A. Elsayed, Phytotoxicity of Silver Nanoparticles on *Vicia faba* Seedlings, *Science*, **6,** 12 (2013).
31. S. S. Hojjat, Impact of Silver Nanoparticles on Germinated Fenugreek Seed, *Int. J. Agri. Crop Sci*., **8**, 627 (2015).
32. A. Parveen and S. Rao, Effect of Nanosilver on Seed Germination and Seedling Growth in *Pennisetum glaucum, J.Clust. Sci*., **26**, 693 (2015).
33. A. Razzaq, R. Ammara, H.M. Jhanzab, T. Mahmood, A. Hafeez and S. Hussain, A Novel Nanomaterial to Enhance Growth and Yield of Wheat, *J. of Nanoscience and Technol.*, ***2****, 55–58(2016).*
34. C. Krishnaraj, E.G. Jagan, R. Ramchandran, S.M. Abirami, N. Mohan and P.T. Kalaichelvan, Effect of biologically synthesized silver nanoparticles on *Baccopa monnieri (Linn.*) Wettest. plant growth metabolism, *Process biochem.* **47**, 651 (2012).
35. D. Stampoulis, S. K. Sinha and J. C. White, Assay-Dependent Phytotoxicity of Nanoparticles to Plants, *Environm. Sci. Technol.* **43**, 9473 (2009).
36. Y. S. El-Temsah and E. J. Joner, Impact of Fe and Ag nanoparticles on seed germination and differences in bioavailability during exposure in aqueous suspension and soil, *Environ Toxicol.* **27**, 42 (2012).
37. M. Sardar and M. Gupta, Silver Nanoparticles and Its Effect on Seed Germination and Physiology in Brassica juncea L. (Indian Mustard) Plant, *Adv. Sci. Lett*. **20**, 1676 (2014).
38. M. Wierzbicka and J. Obidzinska, The Effect of Lead on Seed Inhibition and Germination in Different Plant Species, *Plant Sci*., **137**, 155 (1998).
39. Z. M. Xiu, J. Ma and P. J. Alvarez, Differential Effect of Common Ligands and Molecular Oxygen on Antimicrobial Activity by Silver Nanoparticles Versus Silver Ions, *Environ. Sci. Technol*., **45**, 9003 (2011).
40. L. Zheng, F. Hong, S. Lu and C. Liu, Effect of Nano-TiO2 on Strength of Naturally Aged Seeds and Growth of Spinach, *Biol. Trace Elem. Res*., **104**, 82 (2005).
41. C. M. Lu, C. Y. Zhang, J. Q. Wen, G. R. Wu and M. X. Tao, Research of the Effect of Nanometer Materials on Germination and Growth Enhancement of *Glycine max* and its Mechanism, *Soybean Sci*., **21,** 168 (2002).

42. Z. Lei, S. Mingyu, W. Xiao, L. Chao, Q. Chunxiang and C. Liang, Antioxidant Stress is Promoted by Nano-anatase in Spinach Chloroplasts Under UV-B Radiation, *Biol. Trace Elem. Res*., **121**, 69 (2008).
43. J. Liuand R. H. Hurt, Ion Release Kinetics and Particle Persistence in Aqueous Nano-silver Colloids, *Environ. Sci. Technol*., **44**, 2169 (2010).
44. Q. L. Feng, J. Wu, G. Q. Chen, F. Z. Cui, T. N. Kim and J. O. Kim, A Mechanistic Study of the Antibacterial Effect of Silver Ions on *Escherichia coli* and *Staphylococcus aureus*, *J. Biomed. Mater. Res*., **52,** 662 (2000).
45. M. Yamanaka, K. Hara and J. Kudo, Bactericidal Actions of a Silver Ion Solution on *Escherichia coli*, Studied by Energy-filtering Transmission Electron Microscopy and Proteomic Analysis, *Appl. Environ. Microbiol*., **71**, 7589 (2005).
46. G. McDonnell and A. D. Russell, Antiseptics and Disinfectants: Activity, Action, and Resistance *Clin. Microbiol. Rev*., **12**, 147 (1999).
47. J. R. Morones, J. L. Elechiguerra, A. Camacho, K. Holt, J. B. Kouri, J. T. Ramirez and M. J. Yacaman, The Bactericidal Effect of Silver Nanoparticles, *Nanotechnol*., **16**, 2346 (2005).
48. U. Samuel and J. P. Guggenbichler, Prevention of Catheter-related Infections: The Potential of a New Nano-silver Impregnated Catheter, *Int. J. Antimicrob. Agents*, **23**, S75 (2004).
49. E. T. Hwang, J. H. Lee, Y. J. Chae, Y. S. Kim, B. C. Kim, B. I. Sang and M. B. Gu, Analysis of the Toxic Mode of Action of Silver Nanoparticles Using Stress-specific Bioluminescent Bacteria, *Small*, **4,** 746 (2008).
50. K. Lamsal, S. W. Kim, J. H. Jung, Y. S. Kim, K. S. Kim and Y. S. Lee, Application of Silver Nanoparticles for the Control of *Colletotrichum* Species *In Vitro* and Pepper Anthracnose Disease in Field, *Mycobiol*., **39**, 194 (2011).